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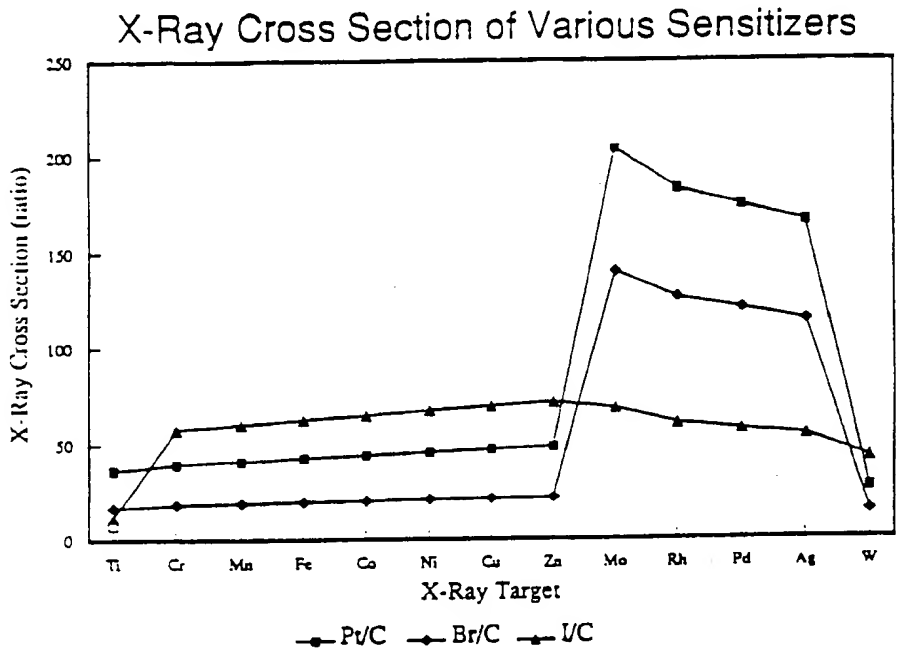

(57) Abstract

A method is provided for inactivating viral and/or bacterial contamination in blood cellular matter, such as erythrocytes and platelets, or protein fractions. The cells or protein fractions are mixed with chemical sensitizers and irradiated with, for example, gamma or X-ray radiation.

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METHOD OF INACTIVATION OF VIRAL AND BACTERIAL BLOOD CONTAMINANTS

This is a continuation-in-part of Serial No. 07/685,931, filed April 16, 1991, which is a continuation-in-part of Serial No. 07/656,254, filed February 15, 1991, which is a continuation-in-part of Serial No. 07/632,277, filed December 20, 1990, which is a continuation-in-part of Serial No. 07/510,234, filed April 16, 1990.

FIELD OF THE INVENTION

This invention relates to the general field of biochemistry and medical sciences, and specifically to inactivating viral/bacterial contamination of liquid, lyophilized, reconstituted blood cell compositions comprising erythrocytes, platelets, etc, or protein fractions.

BACKGROUND OF THE INVENTION

A major concern in the use of stored or donated homologous blood or plasma protein preparations derived from human blood is the possibility of viral and bacterial contamination.

Viral inactivation by stringent sterilization is not acceptable since this could also destroy the

functional components of the blood, particularly the erythrocytes (red blood cells) and the labile plasma proteins. Viable RBC's can be characterized by one or more of the following: capability of synthesizing ATP; cell morphology; P_{50} values; oxyhemoglobin, methemoglobin and hemichrome values; MCV, MCH, and MCHC values; cell enzyme activity; and in vivo survival. Thus, if lyophilized then reconstituted and virally inactivated cells are damaged to the extent that the cells are not capable of metabolizing or synthesizing ATP, or the cell circulation is compromised, then their utility in transfusion medicine is compromised.

There is an immediate need to develop protocols for the deactivation of viruses that can be present in the human red blood supply. For example, only recently has a test been developed for Non A, Non B hepatitis, but such screening methods, while reducing the incidence of viral transmission, do not make the blood supply completely safe or virus free. Current statistics indicate that the transfusion risk per unit of transfused blood is as high as 1:100 for Non A, Non B hepatitis, and ranges from 1:40,000 to 1:1,000,000 for HIV, depending on geographic location. Clearly, it is desirable to develop a method which inactivates or removes virus indiscriminately from the blood.

Contamination problems also exist for blood plasma protein fractions, such as plasma fractions containing immune globulins and clotting factors. For example, new cases of non A, non B hepatitis have occurred in hemophilia patients receiving protein fractions containing Factor VIII which have been

treated for viral inactivation according to approved methods. Therefore, there is a need for improved viral inactivation treatment of blood protein fractions.

The present invention thus provides a method for the inactivation of viral and bacterial contaminants present in blood and blood protein fractions.

The present invention also provides a method for viral or bacterial decontamination of frozen cells, recombinant protein preparations, blood components including red cells, platelets and leukocytes, stem cells, protein solutions or other frozen compositions intended for subsequent *in-vivo* use such as plasma derived factors. The present invention involves utilization of sensitizers which bind selectively to a viral nucleic acid, coat protein or membrane envelope. The sensitizer is also moiety which can be activated upon exposure to radiation, which may be in the form of ultra-violet radiation, but preferably is in the form of ionizing radiation such as X-rays which can penetrate the sample containing the contamination. While not intending to be limited to a particular theory, in frozen cell or protein containing compositions, some of the water is present in the form of ice but there is also unfrozen water trapped in a highly viscous glassy state. Water with molecules which are present in these glassy states have low mobility and may possibly form hydroxy radicals which can randomly damage cells. However, due to the low mobility in the glassy state, damage to cells from these hydroxy radicals is reduced. Therefore, by irradiating a frozen suspension of cells containing the sensitizers, random damage of

the cells due to the hydroxy radicals may be avoided due to the inability of the sensitizer to migrate in the frozen suspension and the inability of the hydroxy radicals to form and migrate through the frozen suspension. In this manner, damage is localized on the targeted viral or bacterial particle.

The present invention also provides a method of using chemical sensitizers and radiation treatment thereof for cytotoxic applications to tumor and cancer cell therapy. Heretofore, four classes of radio sensitizing compounds have been utilized, in various combinations, with radiation to treat tumors. Hypoxic compounds such as molecular oxygen and perfluorinated chemicals are in this category. Thiol-depleting agents such as diamide and diethylmalate inhibit the normal cellular repair of damage caused by chemical radicals by interfering with cell glutathione levels. Metabolic inhibitors such as arabinofuranosyladenine inhibit the cell repair mechanisms that are activated under conditions of low nutrients. Pyrimidine analogs such as halogenated derivatives, deoxyuridine are utilized which are incorporated into the DNA of dividing tumor cells. This substituted DNA becomes more sensitive to radiation damage. Experiments *in vitro* show that when 30% to 40% of the thymidine and DNA is replaced with BUdR or IUdR, there is up to a factor increase in efficiency of radiation damage. Bromodioxyuridine (BUdR) although it is a halogenated compound, acts by a mechanism requiring mitotic cells to incorporate it into new DNA strands. But the halogenated dioxyuridines have not been utilized for viral inactivation. A recent review of use of radio

sensitizing compounds for the treatment of tumors is provided in "Cancer: Principles and Practice of Oncology" second edition, J.B. Lippincott Company, V.T. DeVita, Jr., S. Hellman and S.A. Rosenberg, editors, 1985, pp. 2256-2279.

SUMMARY OF THE INVENTION

The present invention provides a method for viral/bacterial inactivation of dried (lyophilized or evaporatively dried), frozen, liquid or reconstituted cells (erythrocytes, platelets, hemosomes and other cellular or cell-like components) or blood protein fractions, which allows for the cells or protein fractions to be useful in a transfusable state, while still maintaining relatively high cell viability, ATP synthesis and oxygen transport, in the case of cellular components, and therapeutic efficacy, in the case of protein fractions.

The lyophilization and reconstitution media according to the present invention may be utilized to lyophilize and reconstitute proteins, particularly, blood plasma protein fractions. The protein fraction may be virally/bacterially deactivated by mixing with a chemical sensitizer, lyophilized (freeze-dried) or frozen, then irradiated. If the lyophilization media of the invention is used, it is contemplated that the constituents of the media also serve to provide some degree of protection of the dry proteins during irradiation.

A preferred embodiment comprises reducing viral and bacterial contamination of dried or reconstituted cells with washing solutions containing a polymer or mixture of polymers having a molecular weight in the

range of about 1K to 360 K, followed by one or more additional wash cycles using a wash of a dextrose-saline solution at a pH in the range of about 7.0-7.4. The dextrose-saline solution will also contain a polymer having a molecular weight in the range of about 1K to 40K, and preferably about 2.5K. The composition of reconstituted cells will also preferably contain a monosaccharide.

Preferably the cells will have been previously lyophilized using a lyophilization solution buffered in the range of pH of 7.0 to 7.4 preferably by a phosphate-buffered solution. A typical phosphate-buffered lyophilization solution will comprise mono- and di-basic potassium and sodium phosphate (usually in the range of 1-10 mM each) and 5-10 mM adenine. This solution maintains the pH at around 7.2.

A preferred phosphate-buffered solution to be used as the lyophilization buffer will comprise nicotinic acid, reduced glutathione, glutamine, inosine, adenine, monopotassium phosphate, magnesium chloride disodium phosphate all of which will serve as a basic salt buffer at a pH of about 7.2. In addition this lyophilization buffer will contain a final concentration of about 26% weight by volume of a monosaccharide, preferably 1.7 M glucose, and a final concentration of about 3.0% weight by volume of polyvinylpyrrolidone (average molecular weight of 360K), and a final concentration of about 15% weight by volume of hydroxyethyl starch (average molecular weight of 500K).

The term lyophilization is broadly defined as freezing a substance and then reducing the

concentration of the solvent, namely water, by sublimation and desorption, to levels which will no longer support biological or chemical reactions. Usually, the drying step is accomplished in a high vacuum. However, with respect to the storage of cells and particularly erythrocytes, the extent of drying (the amount of residual moisture) is of critical importance in the ability of cells to withstand long-term storage at room temperature. Using the procedure described herein, cells may be lyophilized to a residual water content of less than 10 weight %, preferably less than 3%, and still be reconstituted to transfusable, therapeutically useful cells. Cells with about 3 weight % water content using this procedure may be stored for up to two weeks at room temperature, and at 4°C for longer than eight months, without decomposition. This far exceeds the current A.A.B.B. standard for refrigerated storage of red blood cells of six weeks at 4°C or less than one day at room temperature without decomposition. These dried cells may be deactivated using a chemical sensitizer described herein.

According to the preferred embodiment of the present invention the washed packed red blood cells are mixed with a chemical sensitizer, then washed to remove excess sensitizer not bound to viral or bacterial nucleic acid, and the treated cells are then lyophilized. The dry cell and sensitizer mixture will then be irradiated, typically with gamma radiation, at an intensity of about 3K-50K rads, for a period of time sufficient to destroy viruses (in particular, the single-stranded or double-stranded RNA/DNA viruses), without any substantial adverse

effect on the recovery and usefulness of the cells. Other wavelengths of electromagnetic radiation such as visible light or X-rays, may be used.

In another preferred embodiment, the chemical sensitizers may be added to liquid protein preparations, then lyophilized and irradiated. Particularly preferred are blood protein preparations, including but not limited to, plasma proteins, blood protein extracts, clotting factors (such as Factors VIII and IX), immune globulins and serum albumin.

Dry (lyophilized) cells or protein fractions may be directly mixed with the chemical sensitizer, then irradiated.

From the foregoing description, it will be realized that the invention can be used to selectively bind a metal atom or a metal atom containing chemical sensitizer to blood-transmitted viruses, bacteria, or parasites. Also monoclonal or polyclonal antibodies directed against specific viral antigens (either coat proteins or envelope proteins) may be covalently coupled with either a metal atom or a metal atom-containing sensitizer compound, thereby increasing the effective cross-section of the contaminant to penetrating or other forms of radiation energy.

Since cell compositions also comprise a variety of proteins, the method of decontamination of cells described herein is also applicable to protein fractions, particularly blood plasma protein fractions, including, but not limited to, fractions containing clotting factors (such as Factor VIII and

Factor IX), serum albumin and/or immune globulins. The viral and bacterial inactivation may be accomplished by treating a protein fraction with a sensitizer as described herein. A protein fraction which has been lyophilized and reconstituted may be sensitized and irradiated to deactivate possible contamination. It is contemplated that liquid and frozen protein fractions may also be decontaminated according to the present invention.

Depending upon the nature of the presumed radiolytic mechanism of the sensitizer reaction with the virus, other types of radiation may be used, such as X-ray, provided the intensity and power utilized is sufficient to inactivate the viral contamination without adverse effect on the cells. Mature human red blood cells and platelets lack nucleic acids, therefore the nucleic acid binding sensitizers selectively target contaminating viruses and bacteria. Although described in connection with viruses, it will be understood that the methods of the present invention are generally also useful to inactivate any biological contaminant found in stored blood or blood products, including bacteria and blood-transmitted parasites. Furthermore, the present may be used to inactivate viruses or viral particles for the preparation of vaccines.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plot of X-ray cross section for various sensitizers described in Example 3.

FIG. 2 is a plot of efficiency of sensitizers with Mo and W targets.

FIG. 3 is a plot of Phi 6 with inactivation according to the procedure of Example 5.

FIG. 4 is a plot viral reduction in plasma according to the procedure of Example 6.

FIG. 5 is a plot of viral reduction in samples described in Example 7.

FIG. 6 is a plot of viral reduction in samples described in Example 8.

FIG. 7 is a plot of residual Factor VII in X-ray irradiated lyophilized plasma.

FIG. 8 is a plot of viral reduction of samples described in Example 9.

FIG. 9 is a plot of residual Factor VIII activity in UV irradiated plasma.

FIGS. 10, 11 and 12 are plots of viral reduction in samples described in Example 10.

FIGS. 13, 14, 15 and 16 are plots of viral reduction in samples described in Example 11.

FIG. 17 is a plot of lambda virus reduction with psoralen sensitizer (with and without bromine) in hydrated plasma in sensitizer concentrations of 0.05, 0.1 and 0.2 mg/ml. The sensitizers are psoralen (S#9) and Br-psoralen (S#8), activated with UV.

DETAILED DESCRIPTION OF THE INVENTION

The cells are preferably prepared by immersing a plurality of erythrocytes, platelets and/or hemosomes, etc. in a physiologic buffered aqueous solution containing a carbohydrate, and one or more biologically compatible polymers, preferably having amphipathic properties. By the term amphipathic it is meant there are hydrophobic and hydrophilic portions on a single molecule. This immersion is followed by freezing the solution, and drying the frozen solution to yield novel freeze-dried erythrocytes containing less than 10%, and preferably about 3% or less by weight of moisture, which, when reconstituted, produce a significant percentage of viable, transfusably useful red blood cells, platelets or hemosomes. Preferred methods of reconstitution of the lyophilized composition are described below. Although described in connection with red blood cells, it will be understood that the methods are generally also useful to lyophilize platelets, hemosomes, and blood protein fractions.

The carbohydrate utilized to prepare erythrocyte, platelet and/ or hemosome compositions according to the invention is biologically compatible with the erythrocytes, platelets or hemosomes, that is, non-disruptive to the cells or hemosome membrane, and one which permeates, or is capable of permeating, the membrane of the erythrocytes, platelets or hemosomes. It is also advantageous to stabilize proteins, especially labile blood proteins, with the carbohydrates during lyophilization and irradiation according to the invention. The carbohydrate may be selected from the group consisting of monosaccharides, since disaccharides do not appear to

permeate the membrane to any significant extent. Monosaccharide pentoses and hexoses are preferred as is a final concentration of from about 7.0 to 37.5 weight % in phosphate buffered saline (PBS) or a phosphate buffered solution, preferably about 26%. Xylose, glucose, ribose, mannose and fructose are employed to particular advantage.

It will be understood that the cells may be lyophilized using other protocols and irradiated as described below. Although viral inactivation will be attained, the advantage of retaining a significant percentage of viable useful red blood cells is lost if the described lyophilization procedure is not followed.

The invention will be hereafter described in connection with erythrocytes (RBC's) but it will be understood it is also applicable to platelets, hemosomes or other blood cell types or biological cells, as well as protein fractions, particularly plasma protein fractions.

The erythrocytes will preferably be prepared from whole blood centrifugation, removal of the plasma supernatant and resuspending the cells in PBS or a phosphate buffered solution or a commercial dextrose-saline solution. This wash cycle may be repeated 2-3 times preferably using a commercial dextrose-saline solution, then the packed cells are diluted with the lyophilization buffer described above so that the final diluted concentration of carbohydrate and polymer are maintained in the necessary ranges.

Alternatively, commercially available packed blood cells may be used, which typically are prepared in CPDA (commercial solution containing citrate, phosphate, dextrose and adenine).

Upon lyophilization to a moisture content of less than 10%, and preferably less than 3%, the lyophilized cells may be maintained under vacuum in vacuum-tight containers, or under nitrogen or other inert gas, at room temperatures for extended periods of time in absence of or without significant degradation of their desirable properties when reconstituted for use as transfusable cells. In using the preferred lyophilization method disclosed herein, a particular advantage of the present invention is that the lyophilized cells may be stored at room temperature for extended periods of time, thus obviating the need for low temperature refrigeration which is required for storing liquid CPDA preserved red blood cells prepared by methods of the prior art. The present invention also obviates the need for very low temperature (-80°C) frozen storage of red blood cells in glycerol.

By using the preferred reconstitution method disclosed herein it is a further advantage that the lyophilized red blood cells may be reconstituted at normal temperatures, i.e. greater than about 4°C up to about 37°C, which corresponds to normal human body temperature, and preferably at room temperature (about 22°C). The reconstitution medium is preferably a solution comprising a polymer or mixture of polymers having a molecular weight of from about 2.5K to 360 K, preferably 5K to about 360K, present in a concentration in the range of about 12 to 30%

weight by volume. This polymer may be the same polymer utilized to lyophilize the red blood cells as described above. Hence the polymers polyvinylpyrrolidone, hydroxyethyl starch, and dextran are particularly preferred and most preferred is polyvinylpyrrolidone (preferably molecular weight about 10K) present in a concentration of about 19% weight by volume in the reconstitution solution. The reconstitution solution will be buffered again typically by phosphate-buffered solution comprising monopotassium phosphate and disodium phosphate as described hereinabove to maintain a pH within the range of about 7.0 to 7.4. The most particularly preferred polymer is polyvinylpyrrolidone of an average molecular weight of about 10K. The most preferred reconstitution buffer will also contain adenosine triphosphate (ATP) in a final concentration of about 5mM.

The polymers may be present in the various solutions from a final concentration of about 3.6K weight % up to saturation, and have a molecular weight in the range of from about 2.5K to about 360K. Preferably, the polymers have molecular weights in the range of from about 2.5K to about 500K, most preferably from about 2.5K to 50K, and are present in a concentration of from about 3.6 weight % up to the limit of solubility of the polymer in the solution. Polymers selected from the group consisting of polyvinylpyrrolidone (PVP) and polyvinylpyrrolidone derivatives, and dextran and dextran derivatives provide significant advantages. Most preferred is the use of polyvinylpyrrolidone (an amphipathic polymer) of average molecular weight in the range of 2.5-360K in an amount in the range of 3-20% weight by

volume in the solution prior to lyophilization. Amino acid based polymers (i.e., proteins), dextrans or hydroxyethyl starch may also be employed. In the lyophilization buffer hydroxyethyl starch (M-HES) with an average molecular weight of about 500K is employed in a 15% weight by volume final concentration. Other amphipathic polymers may be used, such as poloxamers in any of their various forms. The use of the carbohydrate-polymer solution in the lyophilization of red blood cells allows for the recovery of intact cells, a significant percentage of which contain biologically-active hemoglobin.

The most preferred reconstitution buffer will be a solution comprising monopotassium phosphate, disodium phosphate and ATP, all of which form a basic salt buffer at a pH of about 7.2, which also contains about 19% weight by volume of polyvinylpyrrolidone (average molecular weight about 10K).

The reconstitution solution may also optionally contain a monosaccharide, preferably present in the concentration range of about 7.0 to 37.5% weight by volume. The preferred monosaccharides are xylose, glucose, ribose, mannose and fructose.

In the most preferred embodiment, the lyophilized erythrocytes can be reconstituted by mixing with an equal volume of the reconstitution buffer at a temperature of about 37°C and mixed. By "equal" it is meant that the volume is the same as the starting volume prior to lyophilization. After initial reconstitution, the solution is preferably diluted 1:1 with 1-4 additional volumes of the reconstitution

buffer at a temperature of about 37°C with added mixing until fully hydrated.

Then, it is preferred that the rehydrated cells be washed according to the following procedure. It is realized, however, that once the cells are reconstituted with reconstitution buffer they are in a hydrated and useful form, but the combination of washings described hereinafter are preferred, specifically for clinical purposes.

After separating the cells from the reconstitution buffer by centrifugation, the resulting packed cells are preferably resuspended at room temperature in (approximately the volume used in the initial reconstitution) a wash buffer comprising nicotinic acid, inosine, adenine, glutamine, and magnesium chloride, all present at about 0.4-10mM further comprising sodium chloride and potassium chloride each at about 30mM, buffered by 10mM disodium phosphate to pH 7.2. This wash buffer further comprises a monosaccharide, preferably glucose at a concentration of about 20mM, and a polymer, preferably polyvinylpyrroltidone, of a molecular weight 40K and present at a concentration of about 16% weight by volume. Separation by centrifugation completes the first post-rehydration step, a washing step.

After the washing step the rehydrated cells may be suspended in a dextrose-saline transfusion buffer at room temperature which preferably contains polyvinylpyrrolidone at a 10% weight by volume final concentration, with an average 2.5K molecular weight. The cells can be used as is or be returned to

autologous plasma. Additional wash steps in a phosphate-buffered diluent buffer can further remove viruses, but this step is optional for preparation of rehydrated, transfusible cells.

The reconstitution and washings described above will in most instances achieve about 4 log reduction of any viral and bacterial contamination, where 1 log reduction is achieved by drying and 3 log reduction is achieved by washing. Of course, different viruses may respond differently, potentially resulting in more than 4 log reduction of contamination.

The reconstituted cells have characteristics which render them transfusable and useful for therapeutic purposes in that their properties are similar to that of fresh (i.e. not previously lyophilized) red blood cells. Typically reconstituted red blood cells according to the present invention have an oxyhemoglobin content greater than about 90% of that in normal red blood cells. Hemoglobin recovery prior to any washing step is typically in the range of 80 to 85%. The overall cellular hemoglobin recovery including the post-hydration washing steps is about 20 to 30%. The morphology of the reconstituted cells according to the present invention (by scanning electron microscope) typically shows no holes or gaps, and primarily discocytic with some stomatocytic morphology. The oxygen carrying capacity of fresh red blood cells (as measured by P_{50}, the oxygen partial pressure at which 50% of the oxygen molecules are bound) was measured to be in the range of about 26 to 28 (average 26.7); with an average Hill coefficient (a measure of the cooperative binding of oxygen molecules to native hemoglobin) of 1.95. The

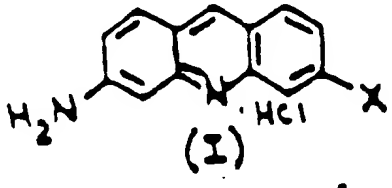
typical P_{50} for erythrocytes lyophilized and reconstituted according to the present invention is about 27.5 (average) with an average Hill coefficient of 2.08. Assays of ATP in the reconstituted cells indicate ATP levels suggesting normal ATP to ADP metabolism. Normal hemagglutination by readily available blood typing antisera of red blood cells made according to the present invention is also typically found.

This lyophilization and reconstitution procedure advantageously and significantly diminishes viral/bacterial contamination in cell-like material (such as hemosomes), and protein fractions. The contamination can be further reduced by the radiation sensitizing and treatment, particularly while the cells or protein fractions are in the dry state.

The starting packed red blood cells or proteins (which may initially be in a liquid or lyophilized state) are mixed with a sufficient amount (based on total wet weight of cells) of a chemical sensitizer. Preferably, in a composition of packed red blood cells (about 10% hematocrit) about 0.1 to 1 mg of the chemical sensitizer will be used per ml of packed cells. Preferably, the mixture will be irradiated with gamma radiation in the range of 3K-50K rads, typically about 3K rads. Preferred exposure is from 1-10 minutes, if using gamma radiation. Alternatively, UV light (320 nm) may be used, particularly for protein fractions. Preferred exposure is from 1-10 minutes, preferably 3 minutes, if using UV radiation. By this irradiation in presence of a sensitizer, there will be about a 6 log reduction of viral and bacterial contamination, based

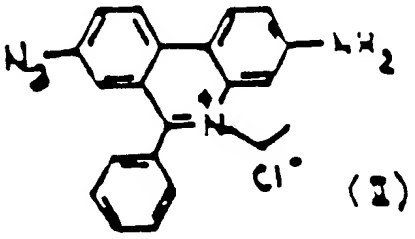
on contamination present prior to washing and irradiation.

The present invention provides a selective method of generating free radicals derived from chemical sensitizers only in the vicinity of viral RNA or DNA. Indiscriminate radiolysis of blood containing virus in a hydrated state produces hydroxyl radical. However, the hydroxyl radical will damage both the red blood cells and associated proteins as well as the viral target. Thus, viral inactivation would be achieved at the sacrifice of red cell viability. Therefore, sensitizers which bind to DNA, RNA, viral coat proteins, and/or viral membranes and which can be selected to generate radicals upon irradiation, are required. Since the radiolysis can be performed in the dry state (preferably less than 10% residual moisture), generation of hydroxyl radicals from water is greatly reduced. In this manner indiscriminate radical damage is further prevented. Exemplary compounds include:



(I)

x = $-N_3$, -I



(II)

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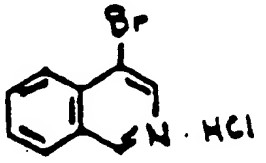

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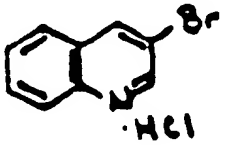


(V)

The preparations of these compounds are known. See Martin, R.F. and Kelly, D.P., Aust. J. Chem., 32, 2637-46 (1979); Firth, W., and Yielding, L.W., J. Org. Chem., 47, 3002 (1982). Other radical-generating reagents which generate radicals upon irradiation are disclosed by Platz et al., Proc. SPIE-Int. Soc. Opt. Eng. 847, 57-60 (1988) and Kanakarajan et al., JACS 110 6536-41 (1988).

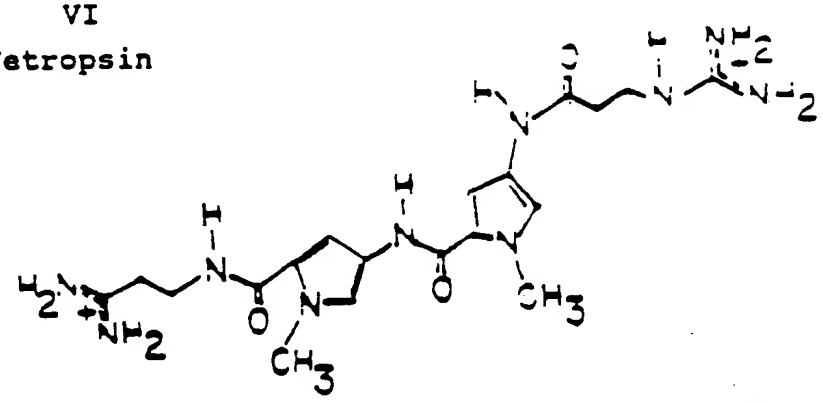
The radiation-sensitizing compound (which may also be modified to bear a metal atom substituent) may also be selected from the class consisting of DNA-binding drugs, including, but not limited to, netropsin, BD peptide (a consensus peptide from HMG-1), S2 peptide, and the like. These and other DNA-binding drugs are disclosed in Pjura, P.E., Grzeskowiak, K. and Dickerson, R.E. (1987), J. Mol. Biol. 197, 267-271; and Tengi, M., Usman, N., Frederick, C.A. and Wang, A.H.J. (1988), Nucleic Acids Res. 16, 2671-2690.

The radiation sensitizing compound (which may also bear a metal atom) can also comprise a class of DNA-binding proteins and/or polypeptides and/or peptides. Examples of this class of DNA-binding proteins and/or polypeptides and/or peptides are disclosed in Churchill, M.E.A. and Travers, A.A. (1991) Trends in Biochemical Sciences 16, 92-97. Specific examples of DNA-binding peptides include the SE peptide and BD peptide disclosed in the reference herein.

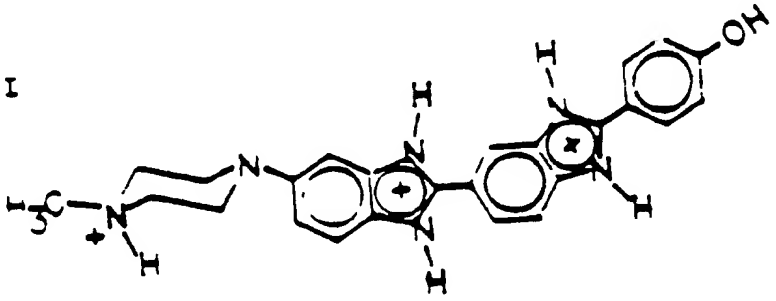
Another class of sensitizers comprises the positively charged porphorins and pthalocyanaies, which bind DNA and RNA. These sensitizers can be activated by irradiation with visible light (500-700 nm).

The DNA-binding specificity can be achieved by covalently coupling the radiation sensitizing compound and/or metal atom to either a DNA-binding drug or to a DNA-binding protein or polypeptide or peptide.

VI
Netropsin



VII



Other sensitizers include specially designed molecules which form triplex DNA, such as those disclosed by Youngquist and Dervan PNAS 82 2565 (1985); Van Dyke and Dervan, Science 225 1122 (1984); Van Dyke and Dervan, Nuc. Acids Res. 11 5555 (1983); Barton and Raphael, PNAS 82 6460 (1985); Barton et al., JACS 106 2172 (1984); and Barton, PNAS 81 1961 (1984). These molecules bind to DNA and RNA, site specifically, if desired, and carry reactive moieties

which can generate free radicals in the proximity of the DNA or RNA.

$$R\text{-}I + e^- \rightarrow R \bullet I^-$$

$$R\text{-}I^{+\bullet} + \text{Guanine} \rightarrow R\text{-}I + \text{Guanine}^{+\bullet}$$

While not intending to be bound by a theory, it is believed that the ejected electron will be captured by that site with the most favorable electron affinity, which is most likely a second molecule of sensitizer elsewhere in the sample. Electron capture by R-I (or R-Br) leads to dissociation of RX with the formation of a radical. The radical so generated will abstract a C-H hydrogen atom from a sugar moiety of a nearby nucleic acid which in turn will lead to DNA or RNA cleavage and viral inactivation.

The radical cation of the sensitizer ($R\text{-}X^{+\bullet}$) will eventually abstract an electron from that component of the sample with the most favorable oxidation potential. This is most likely guanine. The electron transfer reaction forms guanine radical cation. This substance will react with O_2 upon reconstitution with aerated H_2O. This process also leads to DNA cleavage and viral inactivation. Unreacted material and reaction by-products will be removed during the washing steps involved in the reconstitution of the lyophilized cells (Table 2). This process will also further remove any virus not inactivated by the treatment described above.

Compounds (1) and (2) bind tightly to DNA and RNA by either intercalation and/or by electrostatic interactions between positively charged ammonium ion groups and the negatively charged phosphate groups of

the nucleic acid target. Red blood cells do not contain nucleic acids and accordingly will not bind to such compounds by intercalation.

The best mode for using the invention is to add the sensitizer to potentially contaminated blood solutions, and to expose to gamma radiation or x-rays. Fluid solutions of blood are preferably exposed to 3000 rads, and dried lyophilized solid formulations are preferably exposed to 10,000 rads of radiation. It is known that the red cells will survive these doses of radiation in the absence of a sensitizer. Lyophilized blood can withstand higher dosage levels of radiation than hydrated blood.

The gamma radiation or x-ray will be absorbed primarily by the heavy atom of the sensitizer, which will be bound to viral DNA or RNA. The radiation will ionize the sensitizer as follows:

$$R\text{-}I + \underset{(X\text{-}Ray)}{\gamma\text{-}Ray} \rightarrow R\text{-}I^{+\bullet} + e^{-}$$

In some instances, particularly if the sensitizer and red blood cells are allowed to stand together for more than several minutes, sensitizers may diffuse into the red blood cells prior to lyophilization. Antioxidants such as glutathione (an excellent hydrogen atom donor) may be added to the preparation to augment the red cell defenses against free radical initiated damage. It will be understood that incorporation of the sensitizer into cells will also allow inactivation of intracellular viruses, especially viruses thought to reside inside white blood cells (most packed red blood cell units contain residual white cells), or intracellular blood

parasites, such as malaria parasite which infects red blood cells.

The sensitizers are removed from the reconstituted blood serum or protein fraction by the washing protocol described above for lyophilized cells.

It is preferred that gamma or X-ray radiolysis take place in a dried lyophilized blood (or protein), virus, and sensitizer formulation rather than in a wet, fluid material for several reasons. Firstly, the dry material is less sensitive to radiation and can be exposed to larger doses of γ-rays or other penetrating radiation without damage to red blood cells (Table 1). This increases the extent of radiolysis of the sensitizer. Secondly, sensitizer radicals bound to DNA or RNA in the dry state can not dissociate from the virus due to the lack of diffusion in the solid material. This will force the sensitizer radical to react with viral RNA or DNA. Thirdly, the solid state conditions will enhance hydrogen atom transfer reactions of the sensitizer radical with the viral nucleic acid, perhaps by quantum mechanical tunneling. Fourthly, the reconstitution and washing protocol used with lyophilized blood or protein fraction serves as a means to remove unreacted material or reaction by-products, and further removes any virus not affected by the treatment (Table 2).

Other types of radiation may be used including ionizing radiation in general, such as X-ray radiation. In one embodiment a metal and/or halogen atom may be a substituent on a chemical radiation sensitizer molecule which binds to nucleic acids,

thereby targeting the embodiments such as bacteria, parasites and viruses. Metal and halogen atom substituents of chemical sensitizers for this purpose include Pt, Br, I, Zn, Cl, Ca and F. The X-ray source is preferably a tunable source, so that the radiation may be confined to a narrow wavelength and energy band, if so desired. The tunable feature allows for optimization of energy absorption by the metal atoms, thereby directing the absorbed penetrating radiation energy to the production of radicals by a chemical sensitizer bound to nucleic acid.

A preferred metal to be used as the radiation sensitizer is platinum. Another preferred group comprises the halogens, bromine, iodine, chlorine and fluorine. Based on their increasing ability to interact with impinging X-rays, the order of enhancement of radiation sensitizing is expected to be platinum, which is much greater than bromine, which is much greater than iodine, chlorine and fluorine, all of which are much greater than hydrogen.

Compounds containing these atoms, when exposed to X-rays or other forms of ionizing radiation, are capable of forming a reactive species which can interact with the viral nucleic acid, coat protein or lipid envelope, thus destroying it and rendering it non-infectious. This process may be most effective in a dry state where quenching and side reactions due to the presence of water are avoided, however, the procedure will also be applicable in hydrated systems.

In another embodiment of the present invention the sensitizers will be utilized in conjunction with solvent detergent systems. Such detergents are known to decrease the viral titre of plasma or separated plasma fractions, presumably by dissolution of the viruses. Such detergents include, Tween®, sodium cholate, sodium deoxycholate, Triton® and common organic solvents such as ether. Reduction of viral titre by use of these solvent detergents is described for example by Horowitz, *et al.*, Transfusion, 25, (6), 516-522 (1985), and 25, (6), 523-527 (1985); and U.S. Patent No. 4,946,648. The level of reduction by such solvent detergents may vary as reported in literature to a reduction of one log to greater than five logs of viral titre for such viruses as VSV, Sindbis, and Sandai. The present invention may enhance the reduction of viral titre by these solvent detergents when used in conjunction with the sensitizers and exposure to radiation as set forth herein. While not intending to be bound by a theory, it is believed that the solvent detergents act on the viral proteins or lipid membranes to denature or alter them in a manner which makes them more susceptible to the actions of the sensitizers through the changes induced by the detergents.

A particularly preferred class of sensitizers comprise DNA intercalators, such as hydroxyl, amino methyl, or methyl substituted psoralens, which may be added to plasma or plasma fractions followed by UV radiation to reduce the viral contamination therein. The substituted psoralens are described in U.S. Patent No. 4,727,027 wherein a reduction of about 4 to 7 logs of viral contamination was obtained with extended exposure to ultraviolet radiation. The

proposed mechanism of action is to form a photoadduct between the sensitizer and the DNA or RNA of viral origin, which results in loss of infectivity of the virus. According to the present invention, the reduction of viral contamination can be unexpectedly reduced by utilizing brominated psoralens or other halogenated psoralens. For example, it was observed that the bromopsoralens are about 200,000 times more effective in reducing viral activity when compared to use of their non-brominated counterparts. While not intending to be bound by any theory, it is believed that the mechanism of action of the brominated psoralens may be a free radical generation in the proximity of the DNA or RNA resulting in damage of vital nucleic acids of viruses.

The brominated psoralens are in an improvement over the known psoralens and other substituted psoralens when used as sensitizers because the brominated psoralens are an improvement because only one photon of light is required to activate the brominated sensitizer whereas two photons are required to activate a non-brominated sensitizer. Secondly, a brominated psoralen is effective in virtually every intercalative site, whereas a non-brominated sensitizer is effective only in intercalation sites containing a uracil or thymine on different strands of the DNA or RNA. Thirdly, the brominated psoralens may be activated by X-rays as well as UV light.

The use of the brominated or halogenated psoralens is particularly useful in activation in hydrated systems such as plasma, immune sera, tissue culture media containing animal serum or serum components (such as

fetal calf serum), or recombinant products isolated from tissue culture media.

Other types of intercalators may be utilized besides the psoralens and substituted psoralens such as those listed below. These intercalators may be used to target viruses or other blood contaminants, or cancer cells. Thus, halogenated or metal atom-substituted derivatives of the following compounds may be utilized as sensitizers:

dihematoporphyrin esters
hematoporphyrin derivatives
benzoporphyrin derivatives
hydrodibenzoporphyrin dimaleimade

hydrodibenzoporhyrin
dicyano disulfone
tetracarbethoxy hydrodibenzoporhyrin
tetracarbethoxy hydrodibenzoporhyrin dipropionamide

The above compounds in their non-halogenated or non-metal atom substituted forms are disclosed in U.S. Patent Nos. 4,649,151, 4,866,168, 4,883,790, 5,053,423 and 5,059,619, incorporated by reference herein. When modified with halogen atoms or metal atoms, the above-identified classes of compounds may be sensitized with electromagnetic radiation, including visible light.

The present invention may be applied to treatment of tumors as well as liquid blood in *ex vivo* irradiation, such as by methods and apparatus described in U.S. Patents 4,889,129 and 4,878,891.

The above compounds are included in a class named lipophilic dyes which include dyes such as merocyanine 540 and phthalocyanine derivatives. Merocyanine 540 has been disclosed as useful for the treatment of cancer and viral inactivation of blood cells and plasma proteins (Sieber, *et al.*, Photo Chem. and Photo Biology, 46 707-711 (1987)). Phthalocyanine derivatives and other lipophilic dyes are known to bind to the membranes of cancer cells or enveloped viruses. When these compounds are activated with suitable wavelength of electromagnetic radiation, they produce singlet molecular oxygen (Kalyanaraman, *et al.*, PNAS, 84 2999-3003 (1987)), which damages the membrane resulting in the killing of the cancer cells or in viral inactivation. With the addition of radiation sensitizer atoms (metal atoms or halogens) according to the present invention, use of these compounds in combination with suitable radiation produces free radicals in the proximity of the DNA/RNA/viral membranes, when then results in destruction of the viral membranes or nucleic acid to inactivate the virus.

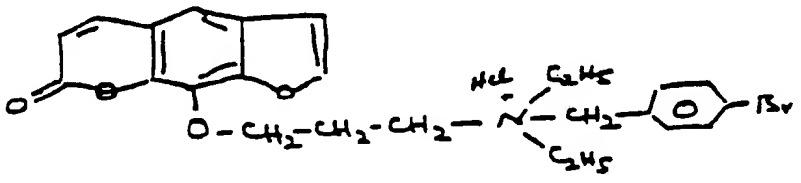
Halogenated or metal atom substituted fatty acids also may be utilized according to the present invention as radiation sensitizers. Fatty acids *per se* have been used in viral inactivation by Horowitz, *et al.* as disclosed in U.S. Patent No. 4,841,023. According to the present invention, these fatty acids may be utilized with sensitizer atoms to target viral membranes in plasma protein solutions, for example, and by subsequent activation with suitable radiation the free radicals are produced to inactivate the viral membrane.

Treatment of biological compositions with detergents are disclosed in U.S. Patent Nos. 4,820,805 and 4,764,369. The clinical application of psoralens in conjunction with photodynamic treatment is discussed by Adelson, *Scientific American* 50-57 (August 1988).

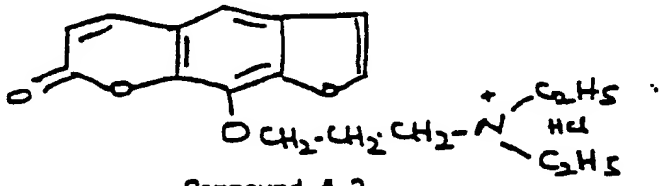
The following compounds are illustrative of sensitizers which contain or which may be modified to contain metal substituents or halogen substituents in accordance with the present invention:

1. DNA or RNA Target Sensitizers

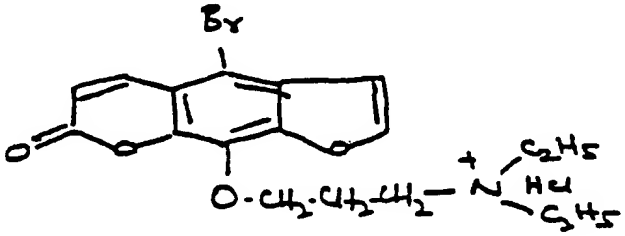
Psoralen Sensitizers:



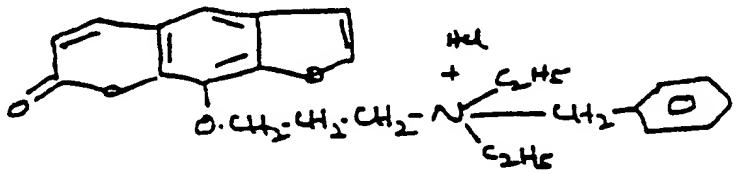
Compound # 1



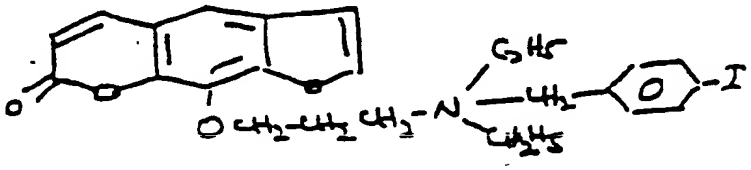
Compound # 2



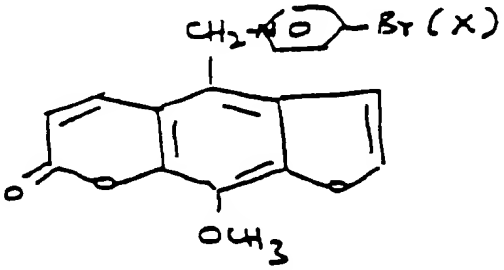
Compound # 3



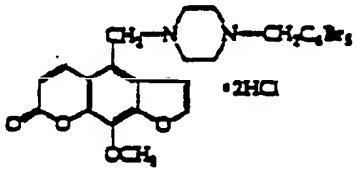
Compound # 4



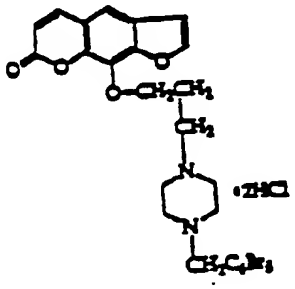
Compound # 5



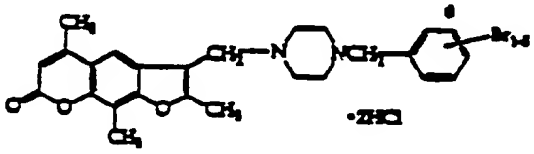
Compound # 6 X=I, Compound 6b



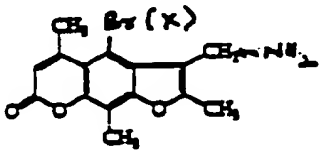
Compound # 7



Compound # 8

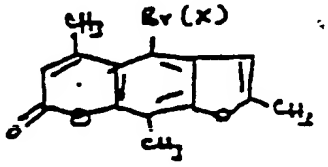


Compound # 9



Compound # 10

X=I, Compound 10b

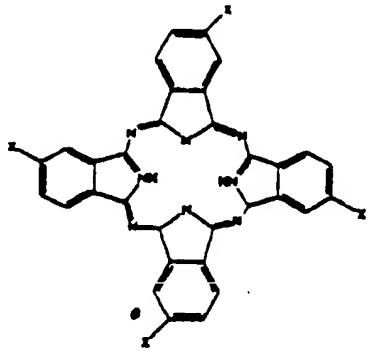


Compound 10c

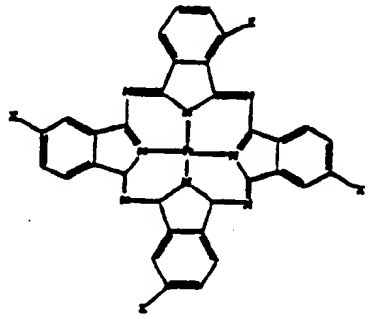
X=I. Compound 10d

2. Membrane Target Sensitizers

Phthalocyanine Sensitizers:

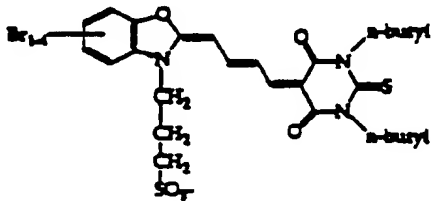


Compound # 11
Compound # 12



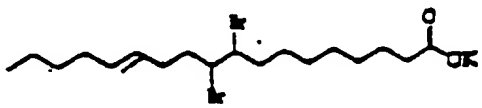
Compound # 13
Compound # 14
Compound # 15

Merocyanine Sensitizers:

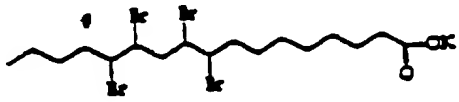


Compound 16

Fatty acid Sensitizers:

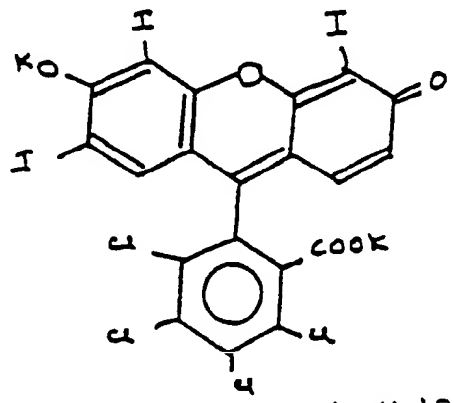


Compound # 17

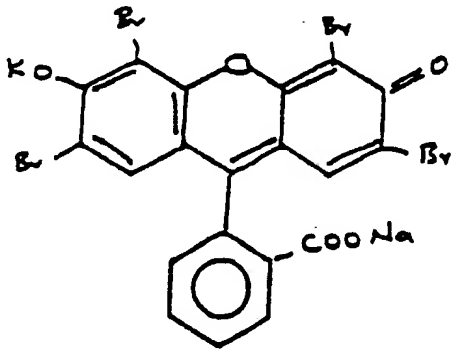


Compound # 18

Biological Dyes:



Compound #19



Compound #20

Other sensitizers include anti-tumor compounds such as doxorubicin and daunomycin, which may be modified with halogens or metal atoms in accordance with the present invention, which are known in chemotherapy to attack dividing cells.

The sensitizers also may be utilized *in vivo* and delivered in liposomes (artificial cells) or drug-loaded natural cells. After introduction of the liposome or drug-loaded cell, the patient may be treated by radiation to activate the sensitizer.

It is within the scope of the present invention to utilize the sensitizers as disclosed herein as cytotoxic agents in combination with chemotherapy with ultraviolet or other ionizing radiation.

The present invention is applicable to contaminants which comprise single or double-stranded nucleic acid chains, including RNA and DNA, and viruses, bacteria or other parasites comprising RNA and/or DNA.

To illustrate the invention, red blood cells were lyophilized as described above, irradiated, and tested for erythrocyte characteristics measured. The results are shown in Table 1. The same procedure was then used, except that the bacteriophage T4 (in dextrose saline) was mixed with the cells and then washed successively with four different wash buffers. The results are shown in Table 2.

Table 1: Influence of irradiation on lyophilized reconstituted red blood cells. Doses as high as 20,000-50,000 rads do not affect cells in the dry state according to the parameters assayed after reconstitution and listed below.

Exposure of Lyophilized Cells to Gamma Irradiation

	* Percentage of Control	
Dosage Level	**20,000 rads**	**50,000 rads**
Hb Recovery	100	99
Oxy Hb	No Change from starting value	No Change from starting value
Cell Indices		
MCV	99	98
MCH	100	100
MCHC	100	100
Metabolism		
ATP (μmol/g Hb)	79	79
Lactate (μmol/g Hb/Hr)	86	79

* Control cells were non-irradiated, lyophilized reconstituted cells.

Table 2: Reduction in viral titre as a function of washing of the red cells. The procedure used in reconstituting the lyophilized cells involves several washing steps which also reduce the viral titre. The extent of reduction with each wash decreases until a practical limit is attained. This represents an approximate 4 log reduction in viral titre.

Washing Protocol Reduction of Viral Load in Blood

Buffer Wash Step	Total Amount of Virus	Log Reduction
Experiment 1 (non-lyophilized cells)		
Reconstitution	7.3 Ox 10^7	0
Wash	4.80 x 10^4	3.2
Diluent	2.08 x 10^4	3.5
Transfusion	3.50 x 10^4	3.3
Experiment 2 (lyophilized cells)		
Lyophilization	3.68 x 108	0
Reconstitution	2.11 x 107	1.2**
Wash	2.38 x 104	4.2
Diluent	2.00 x 104	4.3
Transfusion	4.06 x 104	4.0

In Experiment 1, the effects of lyophilization on viral reduction are not included. In Experiment 2, these effects are included. The marker virus used in these cases was bacteriophage T4. The extent of reduction was determined using the plaque assay.

**This shows an additional about 1 log reduction of contamination due to the drying step.

EXAMPLE 1

Packed human red blood cells purified from donated whole blood are washed free of the anticoagulant storage solution (commercially available CPDA, containing citrate/phosphate/dextrose/adenine), and suspended in dextrose-saline at a 10% hematocrit. Approximately 10 ml of washed packed red cells is placed in a quartz chamber and exposed to U.V. light, preferably at 320 nm, for 2 minute time intervals, up to a 10 minute total exposure. At each 2 minute interval the suspension is mixed and a small sample of red cells (10 microliters) is removed and diluted into 2 ml of water for spectrophotometric assay of hemoglobin. At each step the temperature of the irradiated red cell suspension is measured, to ensure that the suspension did not overheat. At no point did the suspension exceed 26 degrees C (normal body temperature is 37 degrees C). Untreated red cells contain a high proportion of functional oxyhemoglobin (oxyHb), usually in the range of 96% or higher. Oxidation damage can form a semi-stable methemoglobin species (metHb), which can normally be reduced back to oxyhemoglobin by a cellular repair enzyme. Hemichrome represents a more severely damaged form, and can be irreversible. Normal red cells can tolerate a moderate level of methemoglobin. Hemichrome degradation can produce free heme, the iron-porphyrin component of native hemoglobin, which is damaging to cell membranes. Thus it is desirable to minimize hemichrome levels. Each hemoglobin species can be detected at a specific wavelength, using a standard spectrophotometer.

The following data show the sensitivity of the hemoglobin to damage by the increased U.V. exposure.

An exposure of 3 minutes was judged to be usable for viral inactivation using a radiation sensitizer, without inflicting excessive damage to red blood cells.

EXPOSURE (Minutes)	% OXYHB	% METHB	% HEMI
0	96.6	3.4	0
2	90.2	7.5	2.3
4	84.5	13.4	2.1
6	76.7	22.5	0.9
8	72.6	27.4	0
10	66.4	33.6	0

EXAMPLE 2

A suspension (0.1 ml) of bacteriophage lambda or bacteriophage phi-X174, of at least 10EV PFU/ml, is separately added to 4 ml of dextrose-saline containing 1 mg/ml of compounds I or II or III. Each suspension of bacteriophage with a radiation sensitizing compound is then exposed to U.V. radiation of the preferred wavelength (320 nm) in a quartz chamber for the preferred time (3 minutes). A control sample of each bacteriophage suspension, containing a sensitizer, is not exposed to U.V. light. Serial dilutions are performed to quantitate the level of infectious titer, and aliquots of the various bacteriophage samples are then mixed with host bacteria and spread on nutrient agar. Following a normal growth period, the plates are assayed for plaques. Other bacteriophage suspensions are separately irradiated as above, but without added sensitizer, to demonstrate the effect of this dose of U.V. alone.

	Log10 Reduction of Virus Titer	
COMPOUND	phi-X174	Lambda
I ($X=N_3$)	>6.0	>6.0
I (X=I)	4.0	>6.0
II	1.7	>6.0
No compound	2-3	2-3

From these data it can be seen that all three tested compounds significantly increase the sensitivity of double-stranded DNA virus (lambda) to U.V. of the preferred exposure. Compound I is also effective against a single-stranded DNA virus, phi-X174. Compound I is most preferred, showing a high (at least 6 log reduction) inactivation efficacy against both single-strand and double-strand DNA viruses.

EXAMPLES 3

SELECTION OF X-RAY TARGET SOURCE

Refering to FIG. 1, the X-ray cross-section ratios of bromine to carbon, iodine to carbon and platinum to carbon were calculated for their cross-sections in CM^2/G for various X-ray target tubes. Reference: W.H. McMaster, *et al*. UCRL-50174 Section II, Section III, Section IV, and CRC Handbook of Chemistry and Physics, pp. E147, 1979. The data suggest that Mo, Rh, Pd and Ag X-ray target tubes will produce suitable wave length radiation and selectivity in X-ray cross-sections for sensitizer atoms over the carbon element.

EXAMPLE 4

COMPARISON OF CALCULATED RATIOS OF X-RAY CROSS-SECTIONS

FIG. 2 shows the calculated mass attenuation coefficients (X-ray cross-section) of molybdenum and tungsten X-ray targets for cross-sections for iodine,

bromine and platinum sensitizer compounds. The graph indicates that platinum and bromine sensitizer atoms absorb 100 to 175 times more radiation energy than carbon for low atomic number elements using a molybdenum target. However, the selectivity is reduced with a tungsten target. This suggests that the combination of platinum and bromine sensitizer atoms and a molybdenum target tube will allow activation of these sensitizers in the presence of a large excess of cellular and protein material. The viral inactivation obtained with molybdenum X-ray radiation and sensitizers with different heavy atoms are illustrated in FIG. 3.

EXAMPLE 5

VIRUS INACTIVATION IN DRY/WET PLASMA

Stock solution of ϕ 6 phage was added to plasma to obtain the final virus concentrate of 1.2×10^7 PFU/ml. Compounds 19 and 20 (formulas given above) and fluorescein were added to the mixture to give the sensitizer concentration of 0.5 mg/ml. After addition of the sensitizer, the solution was mixed on a mechanical shaker for 1 hour at room temperature. The sample was transferred to a plastic Petri dish (35x10mm) and irradiated in a Pantak HP 160 X-ray unit equipped with a Mo target tube operating at 28 ma and 40 kv settings. Approximate radiation dose delivered was 353 kr. After irradiation the residual viral titre was measured by the plaque method. A phage containing irradiated sample was mixed with suitable phage host bacteria and 3 ml of melted soft agar. The mixture was poured over hard nutrient agar plate. After one day of incubation the lysed area stood out as plaque against the dense background. The plaques were counted with a colony counter. All

samples were treated with X-ray radiation unless otherwise stated. For dry lyophilized state irradiation, the samples were prepared as described above and transferred to 50 ml round bottom flasks, lyophilized on a bench freeze dryer for 16 hours. The dry powder was placed in Petri dishes and X-ray irradiated to 353 kr. The residual viral titre was determined in reconstituted plasma by the plaque method as described above. The results are consistent with the hypothetical calculated selectivities shown in FIG. 2.

EXAMPLE 6

VIRAL INACTIVATION IN HYDRATED PLASMA

The initial titre of ϕ 6 virus 1.2 x 10^7 PFU/ml in compound 20 (structure given above) and 0.5 mg/ml were used in hydrated plasma. The sample preparation and irradiation conditions were as described in FIG 3. For R-17 virus the starting viral titre of 4.2 x 10^8 PFU/ml in compound No. 20 at 0.5 mg/ml were used. The results indicate that enhanced viral reduction is obtained with the use of sensitizers in combination with the X-ray radiation treatment.

EXAMPLE 7

VIRAL INACTIVATION WITH MOLYBDENUM X-RAY RADIATION IN LYOPHILIZED PLASMA WITH VARIOUS RESIDUAL MOISTURE CONTENTS

FIG. 5 shows the inactivation of enveloped and non-enveloped viruses obtained with molybdenum X-ray radiation treatment in lyophilized plasma preparations. The residual moisture content of Sample 1 was about 7.7% and of Sample 2 was about 2.4% as determined by a Karl/Fisher titrater. The starting concentrations of the viruses in the samples were ϕ 6 (2.0 x 10^7 PFU/ml and R-17, 4.4 x 10^8

PFU/ml). Sample 1 and Sample 2 were lyophilized in a Petri dish and round bottom flask respectively. All samples were treated with 353 kr dose and the final virus concentration was determined by the plaque method. The results indicate that the amount of the residual moisture in lyophilized samples significantly influences the degree of inactivation obtained with X-ray radiation treatment in the presence of and absence of a sensitizer.

EXAMPLE 8

VIRAL INACTIVATION IN LYOPHILIZED PLASMA IN THE PRESENCE AND ABSENCE OF A SENSITIZER WITH MOLYBDENUM X-RAY RADIATION

Samples have starting ϕ 6 virus titre of 8.1×10^8 PFU/ml and compound No. 20 (structure given above) at 0.5 mg/ml were used in lyophilized plasma. The radiation dose employed was 420 kr. For the R-17 virus the conditions were: initial titre 3.7×10^8 PFU/ml and compound No. 7 (structure given above) at 0.2 mg/ml, 353 kr radiation dose. Samples not irradiated with X-ray radiation showed very small or no change in starting viral titre value. The results are shown in FIG. 6 where there is shown a 7 log reduction in viral titre of ϕ 6 and a 4.6 log reduction in viral titre of R-17 using a sensitizer. FIG. 7 shows the residual factor H activity X-ray irradiated lyophilized plasma (AHF concentrate) from the same samples. The damage factor to Factor VIII activity in lyophilized plasma during viral inactivation treatment is negligible. Greater than 95% recovery of Factor VIII recovery is shown using the sensitizer.

EXAMPLE 9

VIRAL INACTIVATION IN HYDRATED PLASMA USING UV IRRADIATION TREATMENT

A stock solution of bacterial phage λ is added to plasma to obtain final titre of 1.2×10^8 PFU/ml and compound No. 3 (structure given above) at 0.1 mg/ml concentration. The mixture was transferred to Pyrex glass photolysis cells and exposed to UV (300-360 nm blue lamp) for 5 minutes. After UV irradiation treatment the final viral titre and Factor VIII activity were measured as described earlier. FIG. 8 shows viral reductions obtained. About 3-6 logs of increased viral inactivation was obtained with protein coated viruses using a sensitizer. The recovery of Factor VIII in these samples after inactivation treatments is shown in FIG. 9. Only 10% of Factor VIII activity was recovered after viral inactivation treatment. However, addition of antioxidants, such as vitamin E, increase Factor VIII recovery under UV irradiation.

EXAMPLE 10

VIRAL INACTIVATION IN HYDRATED AND LYOPHILIZED PLASMA IN PRESENCE AND ABSENCE OF ORGANIC SOLVENTS

The starting titre of ϕ 6 virus was 2.5×10^8 PFU/ml in these samples. The plasma mixture was mixed with either 1% Tween 80 detergent or 1% tri-(n-butyl)phosphate (TNBP) for 2 hours at room temperature. The samples were treated with 353 kr radiation dose in hydrated and lyophilized states as described above. The results are shown in FIGS. 10 and 11. The data indicates that the viral inactivation obtained with molybdenum X-ray radiation in hydrated and lyophilized plasma is substantially enhanced by addition of either organic solvent (TNBP)

or detergent (Tween 80) individually or in combination. FIG. 12 shows the results using a stock solution of R-17 virus added to plasma to give a starting titre of 6.2×10^8 PFU/ml and 1% Tween 80 with 1% TNBP for two hours at room temperature. The hydrated and lyophilized samples were treated with 353 kr radiation dose and the final viral titre was determined as described above.

EXAMPLE 11

VIRAL INACTIVATION IN PLATELETS (FROZEN/LIQUID)

Fresh platelets (24 hours old) were spun to remove residual red cells. The platelets were diluted with plasma to obtain approximately 500×10^6 cells ml. The starting titre of ϕ 6 virus in liquid platelet preparation was 2.0×10^5 PFU/ml. After addition of compound 20, 0.3 mg/ml of the sample was transferred to a polyolifin bag (2 inches by 2 inches dimensions) and irradiated to deliver approximately a radiation dose of 195.4 kr. Subsequently the sample was analyzed for morphological evaluation under microscope, cell recovery calculations were made, and aggregation response to collagen (200 µg/ml) was determined by an aggregometer. The final virus titre was determined by the plaque method. The liquid state results were shown in FIGS. 13 and 14. For the frozen samples, fresh platelets 500 x 106 cells/ml were diluted with 10% DMSO in PBS buffer (1:1, v/v). The starting ϕ 6 virus titre was 2.0×10^6 PFU/ml. The compound No. 20 was added at a concentration of 0.3 mg/ml and the sample (2 ml) was transferred to a polyolifin bag and frozen. The frozen bag was treated with 26.3 kr radiation dose. The sample bag temperature was maintained at -80°C using liquid nitrogen during the irradiation treatment. After

irradiation the sample was thawed at 37°C and diluted with PBS slowly to 10 ml. The washed platelets were resuspended in plasma and analyzed for morphological evaluation, cell recovery and aggregation response to collagen (200 mg/ml). The final virus titre after irradiation was determined by thawed samples by the plaque method. Results were shown in FIGS. 15 and 16. These results suggest that the addition of sensitizer increases the viral inactivation by 1 to 2 logs. The irradiation damage to platelets is not significant (70% to 90% control of viability assays) as determined by the morphological score, cell recovery and aggregation response to collagen agonist.

WHAT IS CLAIMED IS:

1. A process for reducing viral, bacterial and/or parasitic contaminants in a composition comprising blood, a blood component, cell culture or a component of a cell culture, comprising the steps of:

mixing said composition in a liquid state with a chemical radiation sensitizer capable of targeting said viral, bacterial and/or parasitic contaminants; and

exposing said composition and sensitizer to electromagnetic radiation of sufficient wavelength and intensity for a period of time sufficient to activate said sensitizer whereby the activation of said sensitizer reduces said contamination in said composition.

2. A process according to Claim 1 wherein, prior to exposing said composition in a liquid state to said electromagnetic radiation, further comprising the step of converting said composition to a solid state.

3. A process according to Claim 2 wherein said solid state is formed by freezing said composition.

4. A process according to Claim 2 wherein said solid state is formed by freeze drying said composition.

5. A process according to Claim 2 further comprising the step of subliming the irradiated composition in the solid state.

6. A method according to Claim 5 further comprising the step of rehydrating the sublimed composition.

7. A process according to Claim 3 further comprising the steps of maintaining said frozen, irradiated solid at temperatures sufficient to maintain the frozen state, and subsequently thawing said frozen solids.

8. A process according to Claim 4 or 5 further comprising the step of rehydrating said composition.

9. A process according to Claims 1 or 2 wherein said composition comprises whole blood or a cellular fraction prepared from whole blood.

10. A process according to Claim 9 wherein said blood cell fraction comprises red blood cells, platelets, white blood cells, or stem cells.

11. A process according to Claims 1 or 2 wherein said composition comprises whole plasma or a blood plasma fraction.

12. A process according to Claim 11 wherein said blood plasma fraction comprises plasma protein fractions.

13. A process according to Claim 12 wherein said plasma protein fractions comprise serum albumin, immune globulins, or a clotting factor.

14. A process according to Claim 13 wherein said clotting factor comprises Factor VIII.

15. A process according to Claim 1 or 2 wherein said cell culture comprises growth media containing serum supplements.

16. A process according to Claim 15 wherein said growth media comprises serum supplements used to propagate mammalian cell lines.

17. A process according to Claim 16 wherein said culture comprises mammalian cell lines containing recombinant genetic material for expression of recombinant proteins.

18. A process according to Claim 17 wherein said culture comprises recombinant plasma proteins.

19. A process according to Claim 18 wherein said recombinant plasma proteins comprise recombinant serum albumin or recombinant clotting factors.

20. A process according to Claim 19 wherein said recombinant clotting factors comprise recombinant Factor VIII.

21. A process according to Claim 16 wherein said mammalian cell lines comprise hybridoma cell lines.

22. A process according to Claim 21 wherein said hybridoma cell lines produce monoclonal antibodies.

23. A process according to Claim 15 wherein said serum supplements comprise whole animal serum or fractions derived from whole animal serum.

24. A process according to Claim 23 wherein said animal serum comprises bovine serum.

25. A process according to Claim 24 wherein said bovine serum comprises fetal calf serum.

26. A process according to Claims 1 or 2 wherein said composition comprises pharmaceutical useful proteins.

27. A process according to Claim 26 wherein said proteins comprise growth factors and hormones.

28. A process according to Claim 1 wherein said electromagnetic radiation comprises ultraviolet light.

29. A process according to Claim 28 wherein said ultraviolet light is characterized by wavelengths of 400 nanometers or less.

30. A process according to Claim 1 wherein said electromagnetic radiation comprises penetrating, ionizing radiation.

31. A process according to Claim 30 wherein said ionizing radiation comprises X-rays or gamma rays.

32. A process according to Claim 1 wherein said electromagnetic radiation comprises visible light.

33. A process according to Claim 31 wherein said X-rays are produced by a metallic target source.

34. A process according to Claim 33 wherein said target source comprises molybdenum.

35. A process according to Claim 33 wherein said target source comprises palladium.

36. A process according to Claim 33 wherein said target source comprises rhodium.

37. A process according to Claim 33 wherein said target source comprises silver.

38. A process according to Claim 33 wherein said target source comprises tungsten.

39. A process according to Claim 33 wherein said target source comprises an element selected from the group consisting essentially of titanium, chromium, manganese, iron, cobalt, nickel, copper, and zinc .

40. A process according to Claim 1 wherein said chemical sensitizer comprises halogenated molecules.

41. A process according to Claim 40 wherein said halogenated molecules comprise bromo-, chloro-, iodo-, or fluoro- derivatives.

42. A process according to Claim 40 wherein said halogenated molecules comprise multiple halogen atoms per molecule.

43. A process according to Claim 1 wherein said chemical sensitizers comprise metal atoms.

44. A process according to Claim 43 wherein said metal atom has an atomic number greater than atomic number = 6.

45. A process according to Claim 44 wherein said metal atom comprises platinum (atomic number = 78).

46. A process according to Claim 40, 42 or 43 wherein said chemical sensitizer comprises halogens and/or metal atoms which increase the overall mass attenuation coefficient of the sensitizer to radiation from a predetermined X-ray target source.

47. A process according to Claim 40, 42 or 43 wherein said chemical sensitizer molecules are derived from membrane binding molecules.

48. A process according to Claim 47 wherein said membrane binding molecules are selected from the group consisting essentially of fatty acid-based molecules and organic dyes.

49. A process according to Claim 47 wherein said membrane binding molecules comprise porphyrins.

50. A process according to Claim 49 wherein said porphyrins comprise hematoporphyrin and hematoporphyrin derivatives.

51. A process according to Claims 40, 42 or 43 wherein said chemical sensitizers are derived from nucleic acid binding molecules.

52. A process according to Claim 51 wherein said nucleic acid binding molecules comprise psoralen and psoralen derivatives.

53. A process according to Claims 40, 42 or 43 wherein said chemical sensitizers are derived from ligands that selectively bind receptor molecules.

54. A process according to Claim 52 wherein said ligands comprise antibodies.

55. A process according to Claim 1 or 2 further comprising the step of treating said composition with organic solvents and/or detergents.

56. A process according to Claim 55 wherein said organic solvent comprises tri-N-butyl phosphate (TNBP).

57. A process according to Claim 55 wherein said detergent comprises a detergent (chemical constituents of Tween 80).

58. A process according to Claim 55 wherein said detergent comprises a nonionic detergent.

59. A process according to Claim 4 wherein said freeze-dried solid is characterized by a residual moisture content of 10% or less.

60. A liquid or solid composition reduced in viral, bacterial and/or parasitic contaminants prepared according to any of Claims 1-7.

61. A composition according to Claim 60 comprising human plasma and/or plasma protein fractions.

62. A composition according to Claim 60 comprising frozen blood cells or frozen blood plasma and plasma proteins.

63. A composition according to Claim 60 comprising liquid, frozen or freeze-dried cell culture media and serum supplements.

64. A substantially viral and/or bacterial and/or parasite-free liquid or solid composition of human blood, blood fractions, or blood products.

65. A substantially viral and/or bacterial and/or parasite free liquid or solid composition comprising tissue culture media containing serum supplements.

66. A process according to Claim 1 wherein said liquid composition containing said chemical sensitizer is irradiated in a blood radiation device.

67. A method according to Claim 66 wherein said device comprises quartz chambers for ex vivo irradiation of blood or blood components using ultraviolet light.

68. A process for selective cytotoxic treatments in vitro comprising:

mixing isolated cells, tissues, or body samples in solution with one or more chemical radiation sensitizers capable of targeting specific subsets of cells, and exposing said mixture to

electromagnetic radiation to activate said sensitizer or sensitizers.

69. A process for selective cytotoxic treatments in vitro comprising:

mixing isolated cells, tissues, or body samples in solution with one or more chemical radiation sensitizers capable of targeting specific subsets of cells;

further comprising the steps of forming solid frozen or freeze-dried matter from said mixture, and exposing said mixture to electromagnetic radiation to activate said sensitizer or sensitizers.

70. A process according to Claims 68 or 69 wherein said chemical sensitizer comprises a halogenated or metal atom containing molecule.

71. A process according to Claim 70 wherein said chemical sensitizer comprises a membrane binding or nucleic acid binding molecule.

72. A process according to Claim 70 wherein said chemical sensitizer is derived from chemotherapeutic agents.

73. A process according to Claim 72 wherein said chemotherapeutic agent comprises dauxorubicin or daunomycin derivatives.

74. A process according to Claim 72 wherein said chemotherapeutic agent comprises a mitotic cell division inhibitor.

75. A process according to Claims 68 or 69 wherein said cytotoxic treatments are used to target and reduce cancer cells in a biological sample.

76. A process for selective cytotoxic treatments in vivo, comprising:

administration in vivo of a chemical radiation sensitizer capable of targeting and binding or being incorporated into selected subset of cells, and exposing said treated tissue or body surface with electromagnetic radiation to activate said sensitizer.

77. A process according to Claim 76 wherein said sensitizer comprises a halogenated or metal atom containing molecule.

78. A process according to Claim 77 wherein said electromagnetic radiation comprises ultraviolet, visible light or ionizing radiation.

79. A process according to Claims 76 or 78 wherein said sensitizer and/or ultraviolet or visible light are delivered to a localized tissue area via a catheter and fiber optic guide.

80. A process according to Claim 79 wherein said delivery area comprises a solid tumor mass or localized cancerous tissue.

FIG 1

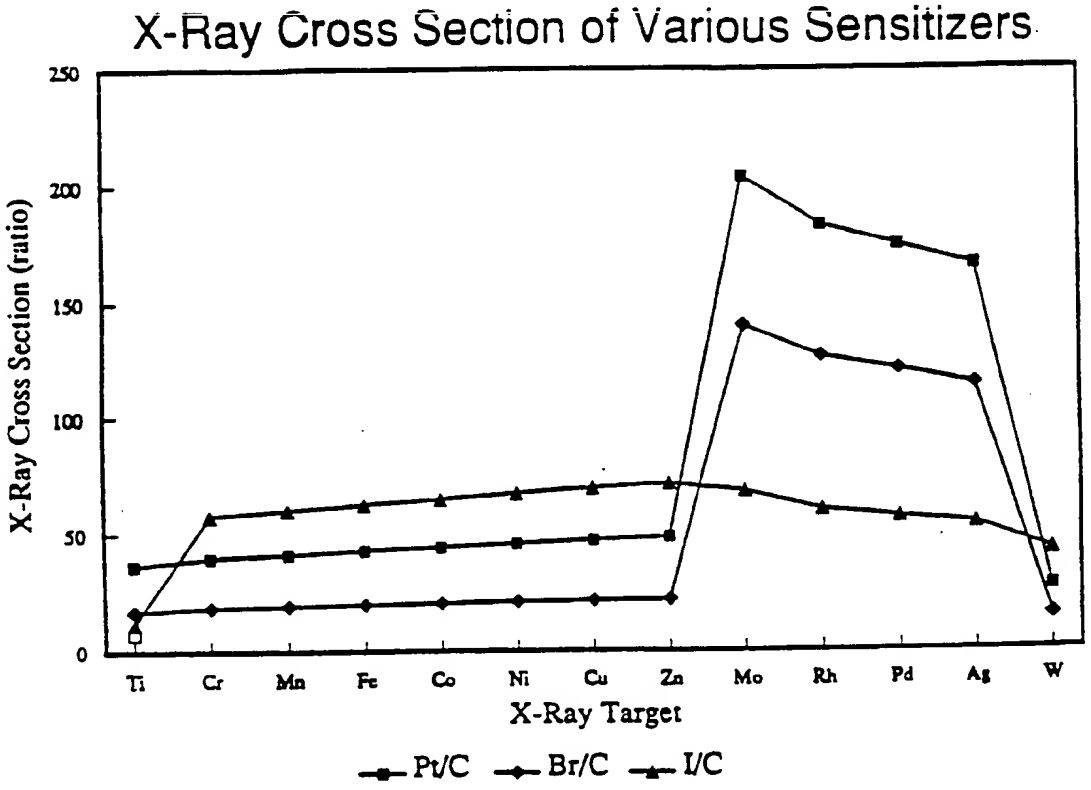
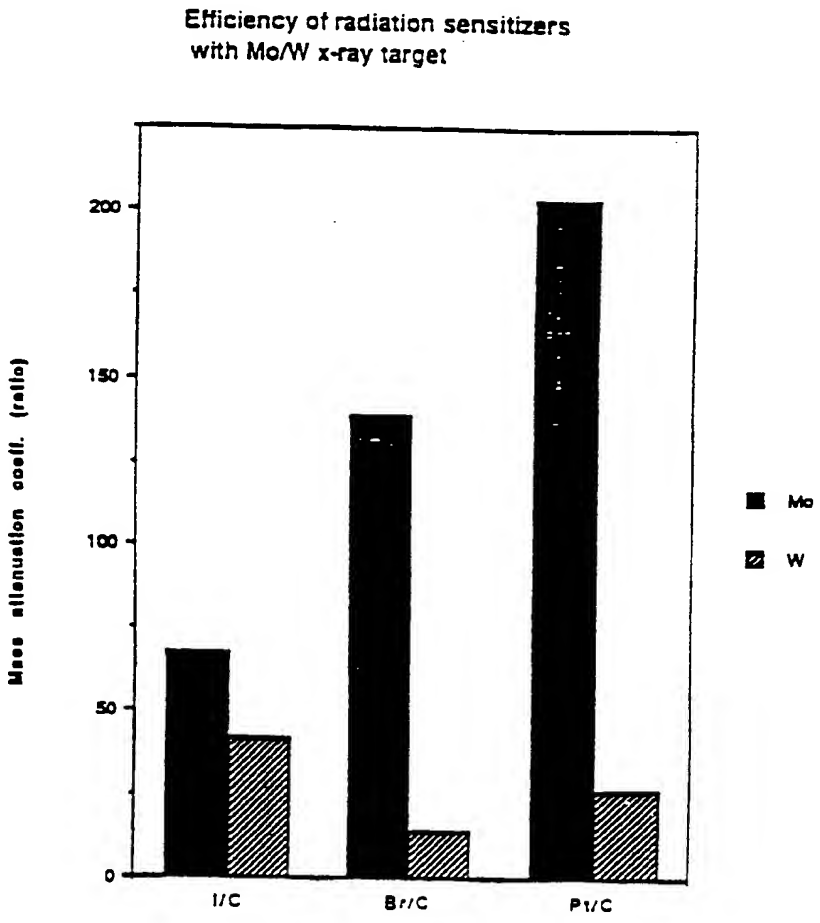

X-Ray Cross Section of Various Sensitizers
X-Ray Cross Section (ratio)
250
200
150
100
50
0
Ti
Cr
Mn
Fe
Co
Ni
Cu
Zn
Mo
Rh
Pd
Ag
W
X-Ray Target
Pt/C
Br/C
I/C

FIG. 2


Efficiency of radiation sensitizers
with Mo/W x-ray target
Mass attenuation coeff. (ratio)
200
150
100
50
0
I/C
Br/C
Pt/C
Mo
W

Phi 6 virus inactivation in dry/wet plasma
(0.5mg/ml sensitizers, 353krads radiation)

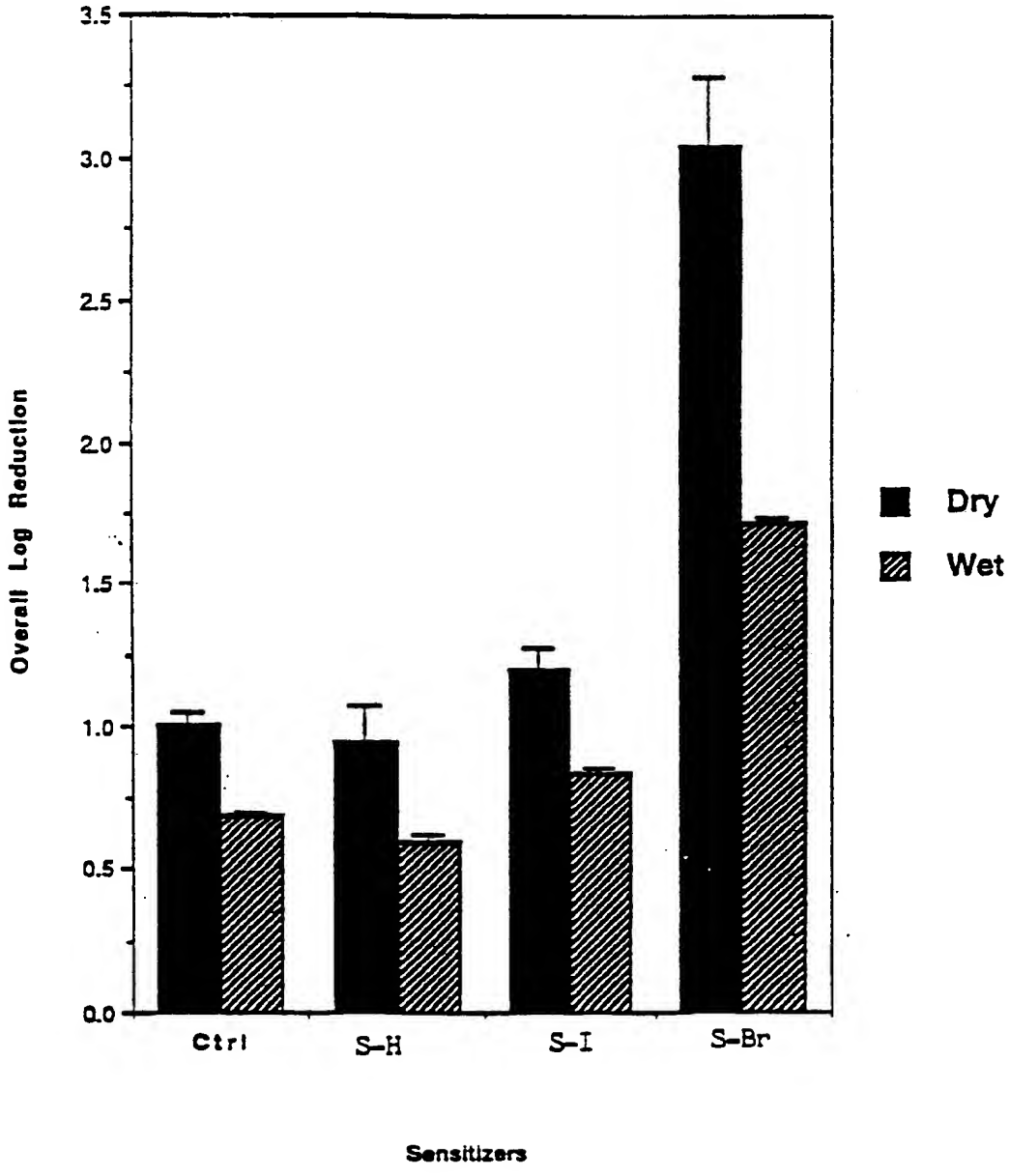


FIG. 4

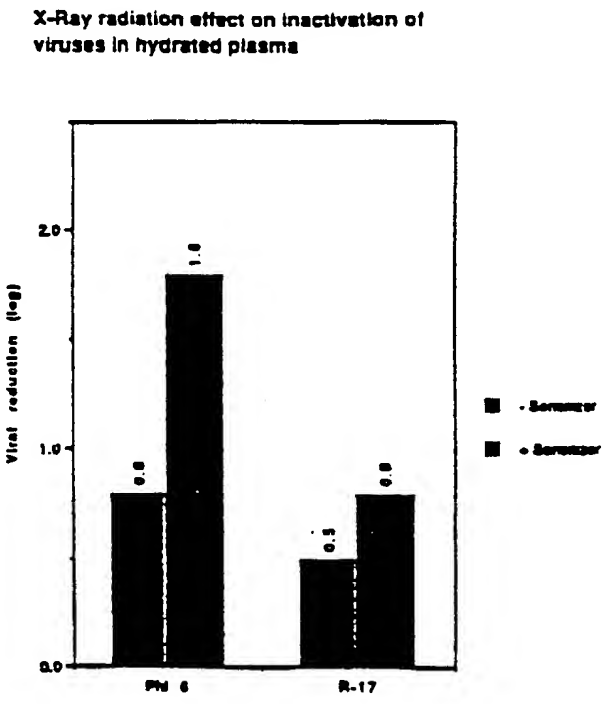

X-Ray radiation effect on inactivation of viruses in hydrated plasma
Viral reduction (log)
2.0
1.0
0.0
0.8
1.8
0.5
0.8
PhI 6
R-17

FIG. 5

Mo. X-ray radiation effect on inactivation of viruses in lyophilized plasma with varying residual moisture contents

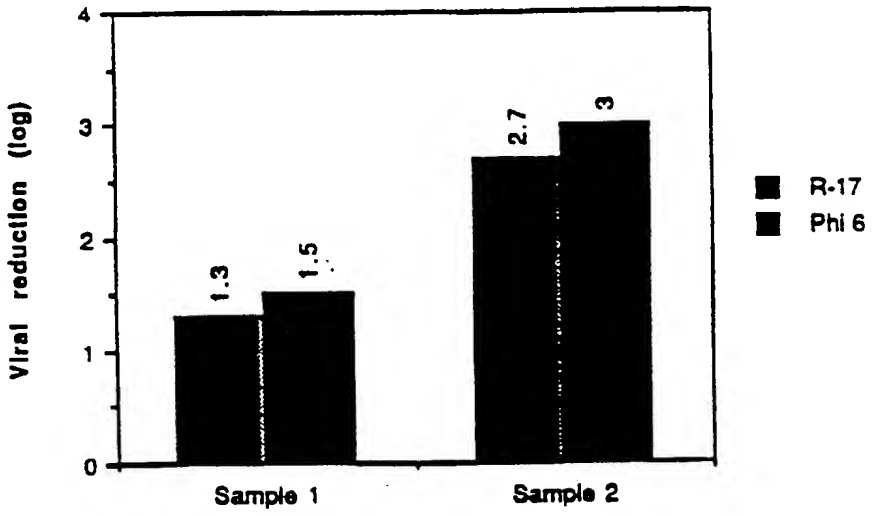


FIG. 6

X-Ray radiation effect on inactivation of viruses in lyophilized plasma

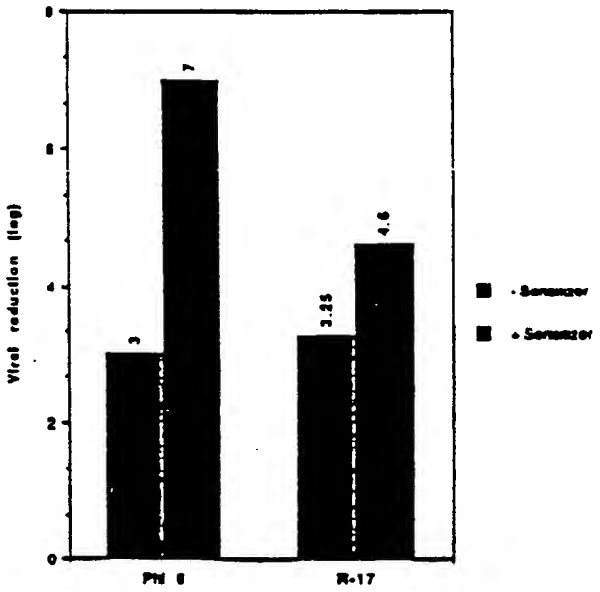


FIG. 7
~~FIGURE 3b~~

Residual factor VIII activity in X-Ray irradiated lyophilized plasma (AHF conc.)

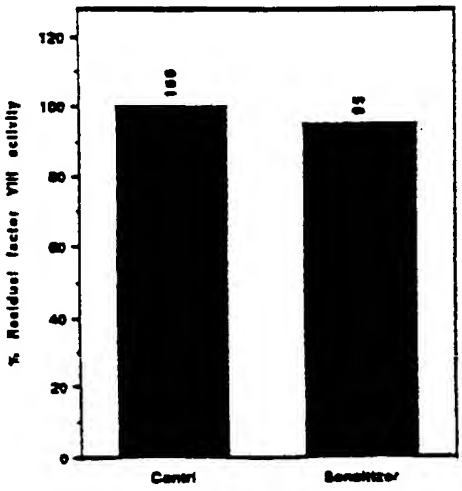


FIG. 8

UV radiation effect on inactivation of viruses in hydrated plasma

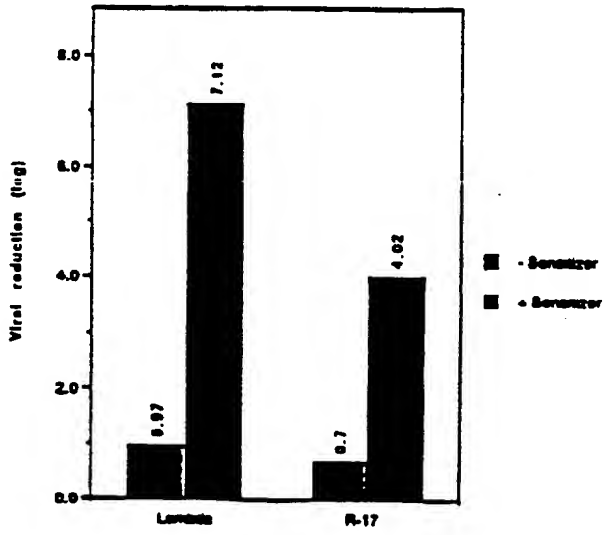


FIG. 9

~~FIGURE 4b~~

Residual Factor VIII activity in UV irradiated hydrated plasma

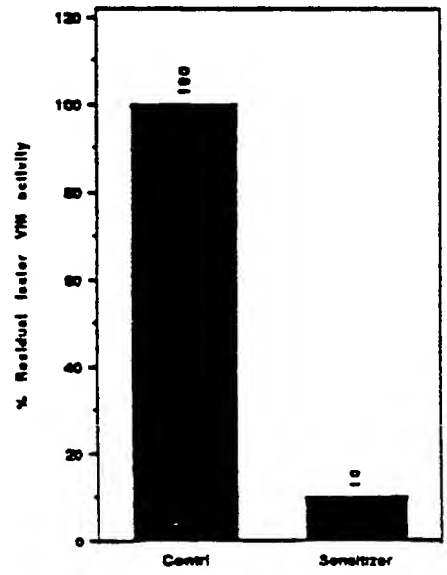


FIG. 13

X-Ray radiation effect on inactivation of viruses in platelets (liquid state)

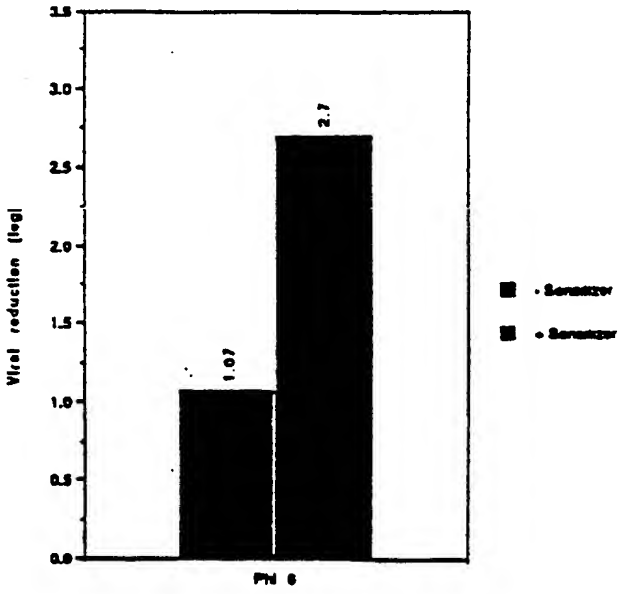


FIG. 15

X-Ray radiation effect on inactivation of viruses in frozen platelets

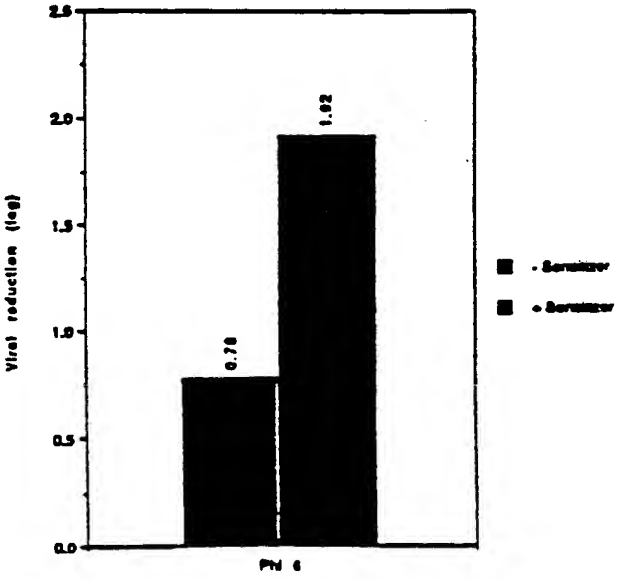


FIG. 14

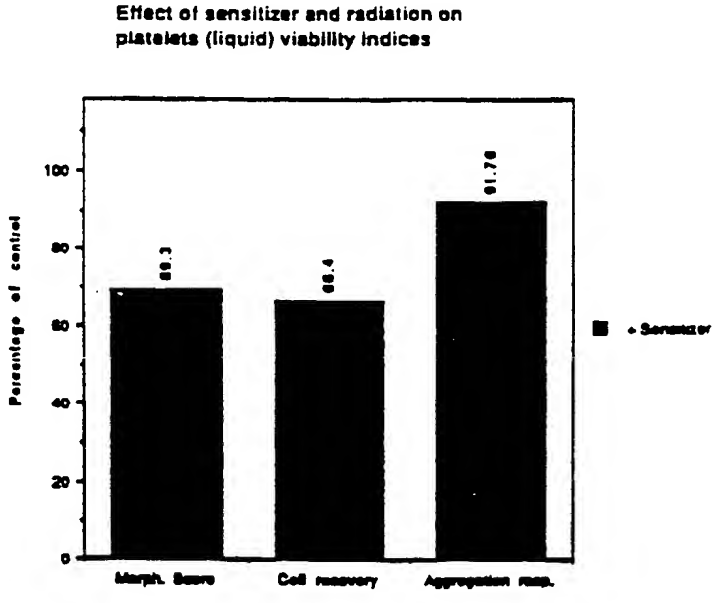

Effect of sensitizer and radiation on platelets (liquid) viability indices
Percentage of control
100
80
60
40
20
0
69.3
68.4
91.78
Morph. Score
Cell recovery
Aggregation resp.
+ Sensitizer

FIG 16

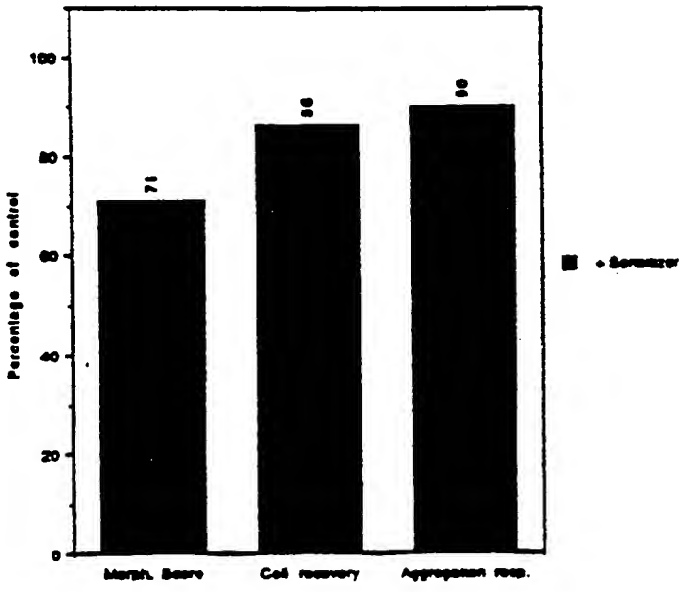

Effect of sensitizer and radiation on frozen platelets viability indices
Percentage of control
100
80
60
40
20
0
71
86
90
Morph. Score
Cell recovery
Aggregation resp.
+ Sensitizer

FIG. 17

Expt: L3. Evaluation of psoralen sensitizer (-/+ Br) against lambda virus in hydrated plasma with UV irradiation (5 min, n=1)